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(54) **Stable aqueous formulations of vinca alkaloids**

(57) This invention provides stable, sterile, ready-to-use formulations of oncolytic vinca dimers comprising an aqueous solution of the vinca dimer (e.g. vincristine, vinblastine, vindesine, 4′-deoxy-1-formyl-leurosidine or leuraformine) as a pharmaceutically acceptable water-soluble salt, an acetate buffer maintaining the pH between 3.0 and 5.0, a polyol (e.g. lactose, mannitol, sorbitol), and a preservative which is preferably methyl paraben and/or propyl paraben.

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SPECIFICATION

Improvements in or relating to formulations of vinca alkaloids

The vinca alkaloids are, in general, dimeric indole-dihydroindole compounds. Two of the alkaloids obtained from the leaves of the plant *Vinca rosea,* vincristine (VCR) and vinblastine (VLB), are marketed for the treatment of leukemias and related neoplasms in humans. A third compound, vindesine (VDS), an amide derivative of vinblastine, is marketed for the treatment of neoplastic diseases in humans in several European countries and is on clinical trial in the United States. These three drugs are described in United States Patents Nos. 3,205,220 (vincristine), 3,097.137 (vinblastine), and 4,203,898 (vindesine). The drugs are administered intravenously to patients suffering from susceptible neoplasms. The usual pharmaceutical formulation employed has been a lyophilized vial of a sulfate salt which is reconstituted prior to use. The sulfate salts are prepared by adding the theoretical amount of sulfuric acid to a solution of the alkaloidal free base. In the case of vindesine, however, the sulfate made by the ordinary procedure is not stable and a special sulfate salt disclosed in U.S. Patent 4,259,242 is employed in the lyophilized pharmaceutical formulation.

Researchers and medical personnel long have thought having ready-to-use solutions of vincristine sulfate or other vinca alkaloids would be desirable. First, improper reconstitution of a lyophilized product sometimes results in the formation of air-borne droplets which mav be a hazard to the hospital personnel who are making up the solution for an i.v. injection. Vincristine is an extremely potent oncolytic drug and avoiding contact with this drug so far as possible is desirable. Furthermore, avoiding all contact with any cytostatic drug and especially vincristine is desirable. In addition, a potential problem always exists during the reconstitution of a lyophilized formulation because an inappropriate quantity of diluent may be used or an incorrent amount of drug may be used because of a different vial size. The margin between toxic effects and therapeutic dose is very small with the vinca alkaloids. Errors in concentrations for i.v. injection resulting in accidential overdosages with vincristine have been recorded in the literature. *See, for example* the *Journal of Pediatrics, 89,* 671 (1976), *Cancer Chemotherapy Reports, 55,* 525 (1972), and *Journal of Pediatrics, 90,* 1042 (1977).

Another disadvantage of lyophilized vincristine sulfate arises from the mode of calculating dose levels for each individual. Vincristine sulfate is supplied in whole milligram amounts (e.g., 1 mg. and 5 mg. vials). Because a dose is usually calculated as 2 mg. per square meter of body surface for children and 1.4 mg. per square meter body surface for adults, the doses actually given are usually in decimal milligram amounts, and therefore only part of a vial's contents may be used. In addition, it should be reiterated that there is a narrow margin between the toxic dose and the effective dose of vincristine. Thus, because the dosage usually is calculated for treating humans, there will ordinarily be some excess of reconstituted vincristine left over after a given treatment. This problem is not particularly serious in a large cancer clinic where there is a daily use of vincristine and that which is left from one patient may be applied to the next. However, the recommended life for reconstituted vincristine is 14 days at refrigerated temperature. Thus, in many instances, discarding excess reconstituted lyophilized vincristine which has outlived its 14 day dating period may be necessary. Vincristine is an extremely expensive drug and any amount of it which must be discarded will increase the overall cost of maintaining a cancer clinic.

Upon standing, the physical changes noticed for reconstituted lyophilized vincristine (reconstituted with 0.9% aqueous sodium chloride containing benzyl alcohol as a preservative) are a general haziness of solution followed by the appearance of a precipitate.

Another problem associated with reconstituted vincristine formulations is the need to incorporate a preservative in order to prevent the growth of micro-organisms. In general, vincristine solutions cannot be heat sterilized but can be sterilized by filtration. However, even if the latter process is used, a preservative must be present in the diluent used to reconstitute the lyophilized material or in an opened previously sterilized liquid vial because of the possibility of contamination from the air. Otherwise, the excess material would have to be discarded immediately and could not be kept even for the recommended maximum 14-day period.

Reconstituted solutions of vinblastine sulfate and vindesine sulfate possess similar problems and concerns although because both compounds contain an N-methyl group instead of the more labile N-formyl functionality found in vincristine, the stability problems are less severe as shown by the recommended reconstituted stability dating of thirty days.

In accordance with the present invention, a stable, ready-to-use solution of oncolytic vinca alkaloids for i.v. injection is provided. The use of these formulations minimizes the contact between hospital personnel and the drug and provides a single solution strength for all vial and syringe sizes employed thereby avoiding error in reconstitution.

In particular, in accordance with the invention, an aqueous pharmaceutical formulation which comprises a pharmaceutically-acceptable vinca dimer salt, a polyol, an acetate buffer, which maintains the pH of the solution between 3.0 and 5.0, and a preservative is useful as a stable oncolytic preparation.

This invention is particularly applicable to the preparation of stable, ready-to-use solutions of vinca dimers, including vincristine sulfate,

vinblastine sulfate and vindesine sulfate. The invention is useful also for the preparation of stable, ready-to-use solutions of certain vinca dimers which are presently on clinical trial or will shortly be on clinical trial as oncolytic agents. Among this second class of compounds are 4'-deoxy-1-formylleurosidine sulfate and leuroformine. This invention may also provide stable, ready-to-use solutions of other vinca dimers whose clinical use is not yet determined but which may be marketed as clinically useful oncolytic agents in the future.

The stabilized formulations of this invention are more applicable to, and useful with, N-formyl vinca dimers such as vincristine or 4'-deoxy-1-formylleurosidine because such compounds decompose by an additional mechanism, *i.e.,* loss of the N-formyl group, not present with vinblastine or vindesine.

Pharmaceutically-acceptable salts other than the sulfate salt, such as the phosphate salt, may be used in the stable solutions of this invention although the sulfate salts are preferred. Pharmaceutically-acceptable salts are those salts useful in the chemotherapy of warm-blooded animals. The alkaloids usually are present in the formulation at a concentration of about 0.01 to 2.0 mg./ml., preferably at a concentration of 0.1 to 1.0 mg./ml.

The polyols useful in these stable, ready-to-use solutions of oncolytic vinca dimers are generally those derived from sugars, such as mannitol and sorbitol, or are sugars themselves such as lactose and sucrose. Other useful polyols will be recognized by those skilled in the art. Lactose and especially mannitol are the preferred polyols use in this invention. The polyol usually is present in the formulation from about 10—100 mg./ml.

The acetate buffer system utilized in these stable solutions should maintain the pH in the range 3.0—5.0. The preferred pH ranges vary with the individual vinca alkaloid. In the case of vincristine sulfate, a pH range of 4.4—4.8 is preferred. For vinblastine sulfate, the preferred range is 3.8—4.2 and for vindesine sulfate, a pH range of 3.0—3.6 is preferred, especially the range 3.2—3.4. A buffer system with a molarity in the range of about 0.0005—0.02 M, preferably 0.002—0.01 *M* is used. The molar ratio of acetate to vinca dimer is preferably about 20 to 1 or less. In the case of vindesine sulfate, it will be recognized by those skilled in the art that, for pH's below 3.6, the "buffer" consists only of acetic acid and no acetate salt is employed. Although at pH 3.0 the acetic acid concentration approaches 0.06 *M*, at the preferred pH range of 3.2—3.4, the acetic acid concentration is about 0.02—0.01 *M*. The stabilizing effect of the acetate buffer may be due in part to preventing a pH change of the solution due to alkali leaching from the glass or stopper of the vial or from degradation due to the change of pH caused by alkaloid decomposition.

In general, preservatives tested in solutions of vinca dimers have had a deleterious effect upon potency, clarity, and pharmaceutical elegance, but, of these, the parabens, methyl and propyl, seem to have little effect on these parameters and are therefore preferred. The parabens may be employed singly or in combination, usually in a total amount of 1—2 mg./ml. Other potential preservatives include benzyl alcohol, phenol, or m-cresol. The liquid formulations produced in this invention are sterilized by filtration.

In addition to the ingredients which are present in these sterile, stable solutions of the alkaloids, the choloride ion concentration should be minimized because chloride ion has a deleterious effect on the various oncolytic vinca dimers.

To further illustrate the invention, the following non-limiting Examples are provided.

Example 1

A stable, ready-to-use solution of vincristine sulfate is prepared as follows: a 1 mg. vial contains vincristine sulfate, 1 mg.; methyl paraben, 1.3 mg.; propyl paraben, 0.2 mg.; mannitol, 100 mg.; acetic acid, 0.0255 ml. of a 0.2 *M* solution; sodium acetate, 0.0245 ml. of a 0.2 *M* solution; water q.s. to 1 ml. Vials containing 2 mg. or 5 mg. of drug are prepared in similar fashion with proportionately larger amounts of materials. The solution thus prepared is sterile filtered and introduced into compatible glass vials in the proper volume. The vials may be purged with an inert gas, such as nitrogen, before the vials are sealed with a compatible stopper.

Alternatively, hypodermic syringes of predetermined volume may be filled with the sterile filtered solution to provide a ready-to-use solution which is also ready to inject. Use of the pre-filled syringe further reduces the chance of exposure to patients or hospital or pharmacy personnel by eliminating the need to transfer a vial's contents to an empty syringe. Ideally, the syringe should be graduated and disposable.

Example 2

An example of a stable, ready-to-use solution of vinblastine sulfate contains the following ingredients: a 10 mg. vial contains vinblastine sulfate, 10 mg.; methyl paraben, 13 mg.; propyl paraben, 2 mg.; mannitol, 1000 mg.; acetic acid, 0.41 ml. of a 0.2 *N* solution; sodium acetate, 0.09 ml. of a 0.2 *M* solution; water, q.s. to 10 ml. The vials are processed in the same way as described above in Example 1. Hypodermic syringes as previously described may be filled with vinblastine solution.

Example 3

An example of a stable ready-to-use solution of vindesine sulfate contains the following: for a 5 mg. vial or syringe, vindesine sulfate, 5 mg.; methyl paraben, 6.5 mg.; propyl paraben, 1 mg.; mannitol, 500 mg.; acetic acid, 0.25 ml. of a 0.2 *M* solution; water, q.s. to 5 ml. The solution may be used to fill vials or syringes as described previously.

Example 4

Ready-to-use formulations of this type must be stable for periods allowing for distribution to the pharmacy and a reasonable shelf life. Vincristine sulfate formulations prepared according to this invention have remained physically and chemically acceptable for pharmaceutical use for periods up to one year at 5°C.

The formulations of this invention were evaluated for their stability by using analytical high pressure liquid chromatography and thin layer chromatography to determine vincristine content and quality. For example, three lots of formulated vincristine sulfate maintained 94—99% of their initial concentration after storage at 5°C. for about nine months. These three lots had the following composition: Vincristine sulfate, 1 mg./ml. of solution; methyl paraben, 1.3 mg./ml. of solution; propyl paraben, 0.2 mg./ml. of solution; mannitol, 100 mg./ml. of solution; acetic acid, 0.0255 ml. of a 0.2 *M* solution per ml. of solution; sodium acetate, 0.0245 ml. of a 0.2 *M* solution per ml. of solution; water, to volume. The solutions were sterile filtered and placed in amber type 1 acid treated vials which were then capped with Teflon®-faced gray-butyl stoppers or Stelmi 632 stoppers. (Teflon® is the registered trademark of E.I. duPont de Nemours & Co., Inc. for polytetrafluoroethylene resins and products). The final pH of the solution was approximately 4.6.

Claims

1. An aqueous pharmaceutical formulation which comprises a pharmaceutically-acceptable vinca dimer salt, a polyol, an acetate buffer to maintain the pH of the solution between 3.0 and 5.0, and a preservative.

2. A formulation according to claim 1 in which the vinca dimer is vincristine, vinblastine, vindesine, 4′-deoxy-1-formylleurosidine or leuroformine.

3. A pharmaceutical formulation as claimed in claim 1 in which the vinca dimer salt is a salt of vincristine.

4. A pharmaceutical formulation as claimed in claim 3 in which the polyol is a sugar or is derived from a sugar.

5. A formulation according to any one of claims 1 to 4 in which the preservative is methyl paraben and/or propyl paraben.

6. A formulation according to any one of claims 1 to 5 in which the concentration of the acetate buffer is 0.0005—0.02 *M*.

7. A formulation according to any one of claims 1 to 6 in which the polyol is mannitol or lactose.

8. A formulation according to any one of claims 1 to 7 containing per ml. of final solution about 1 mg. of vincristine sulfate, 10—100 mg. of mannitol or lactose, 1—2 mg. of a preservative selected from methyl paraben and propyl paraben, singly or in combination, and water q.s. to 1 ml., with a pH of said solution between about 4.4—4.8 maintained by a 0.002—0.01 *M* acetate buffer.

9. A formulation according to claim 8 which contains, per ml. of final solution, about 1 mg. of vincristine sulfate, 100 mg. of mannitol, 1.3 mg. of methyl paraben, and 0.2 mg. of propyl paraben.

10. A formulation according to any one of claims 1, 2 or 5 to 7 containing per ml. of final solution about 1 mg. of vinblastine sulfate, 10—100 mg. of mannitol or lactose, 1—2 mg. of a preservative selected from methyl paraben and propyl paraben, singly or in combination, and water q.s. to 1 ml., with the pH of said solution being between about 3.8—4.2 maintained by a 0.01—0.002 *M* acetate buffer.

11. A formulation according to claim 10 which contains, per ml. of final solution, about 1 mg. of vinblastine sulfate, 100 mg. of mannitol, 1.3 mg. of methyl paraben, and 0.2 mg. of propyl paraben.

12. A formulation according to any one of claims 1, 2 or 5 to 7 containing per ml. of final solution about 1 mg. of vindesine sulfate, 10—100 mg. of mannitol or lactose, 1—2 mg. of a preservative selected from methyl paraben and propyl paraben, singly or in combination, and water q.s. to 1 ml, with pH of said solution between about 3.0 to 3.6.

13. A formulation according to claim 12 which contains, per ml. of final solution, about 1 mg. of vindesine sulfate, 100 mg. of mannitol, 1.3 mg. of methyl paraben, and 0.2 mg. of propyl paraben, with a pH of said solution between about 3.2—3.4.

14. A formulation for vinca dimers as claimed in claim 1 substantially as hereinbefore described with reference to any one of the Examples.

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Degradation kinetics of vincristine sulphate and vindesine sulphate in aqueous solutions

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Summary

The degradation kinetics of the antineoplastic drugs, vincristine and vindesine, have been studied in the pH range from − 2 up to 11 at 80 °C. A stability-indicating HPLC system with UV detection was utilized for the analysis of vincristine and vindesine in the reaction solutions. The influences of external factors (e.g. pH, buffer concentrations, ionic strength and temperature) on the degradation rate have been studied systematically. The relationship between pH and log k_{obs} was modelled by using a non-linear least-squares curve-fitting computer program. From this plot the pK_a values of vindesine have been calculated. This plot also showed that vincristine was most stable at pH 4.8 and vindesine at pH 1.9.

Introduction

Vincristine (VCR) and vinblastine (VBL) (Fig. 1) are naturally occurring Vinca alkaloids, extracted from the *Catharanthus roseus* G. Don. Vindesine (VDS) is a semisynthetic derivative, originating from vinblastine. These cytotoxic agents are in widespread clinical use in cancer chemotherapy for the treatment of haematological as well as solid malignancies (Creasey, 1981). VCR is marketed under the trade name Oncovin, both as a freeze-dried formulation and as a sterile solution for injection. VDS (Eldisine) is commercially available in the lyophylized state only. Both drugs are known to be susceptible to degradation in aqueous and organic solvents (Burns, 1972). Until now, only a few reports on the chemical stability of VCR have been published and most of them were limited (Sethi and Thimmaiah, 1985; De Smet et al., 1985; Beijnen et al., 1986). The information about the stability of VDS is even more scarce (Yang and Drewinko, 1985). For optimal pharmaceutical handling it is of major importance to have a profound insight into the degradation reactions of these cytostatic agents. Therefore, we

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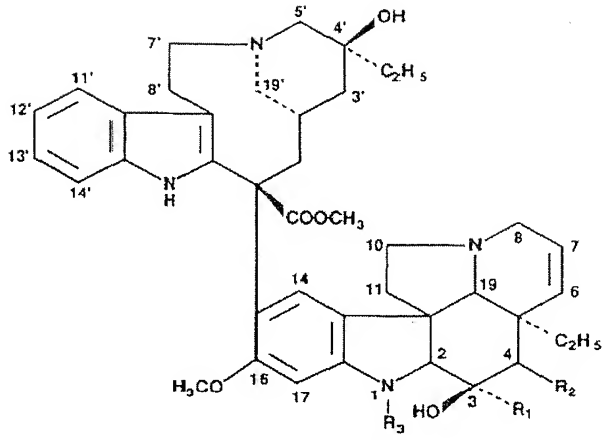

	R_1	R_2	R_3
VINBLASTINE	$COOCH_3$	$OCOCH_3$	CH_3
VINCRISTINE	$COOCH_3$	$OCOCH_3$	CHO
VINDESINE	$CONH_2$	OH	CH_3

Fig. 1. Chemical structures of vinblastine, vincristine and vindesine.

initiated this systematic stability study which is a sequal to an earlier report on the degradation kinetics of VBL (Vendrig et al., 1988a).

Experimental

Materials

Vincristine sulphate was kindly provided by Pharmachemie B.V. (Haarlem, The Netherlands). Vindesine sulphate was a gift from Eli Lilly (Indianapolis, IN, U.S.A.). Desacetylvincristine was a gift from Gideon Richter (Budapest, Hungary). All other chemicals were of analytical grade and were used as received. Deionized water for the preparation of buffer solutions was filtered using a Milli-Q Water Purification System (Millipore, Bedford, MA, U.S.A.).

Buffer solutions

The buffer solutions used in this study were prepared as described previously (Vendrig et al., 1988a).

Kinetic measurements

The degradation kinetics of VCR and VDS were investigated at a temperature of $80 \pm 0.5\,^{\circ}C$. The preparation of the reaction solutions and also the sampling procedures have been described before (Vendrig et al., 1988a). Samples of solutions at extremely low pH values (≤ 0.5) were adjusted to pH 4–5 with a small amount of a sodium acetate solution prior to analysis.

Apparatus and analytical procedures

The chromatographic system consisted of a Spectroflow HPLC pump and a Spectroflow 773 absorbance detector (both from Applied Biosystems, Ramsey, NJ, U.S.A.) operating at 254 nm. A Model 440 dual wavelength absorbance detector (Waters Assoc., Milford, MA, U.S.A.) with fixed wavelength filters of 254 and 280 nm was used to determine the purity of the VCR and VDS peaks. Samples of 10 or 20 μl were injected with a Waters Intelligent Sample Processor (WISP model 710) or by using a U6K valve injector (Waters Assoc.). The separation of the parent drug and the degradation products was accomplished on a Hypersil ODS column (100×3.9 mm i.d., particle size 5 μm). The mobile phase comprised methanol and 10 mM sodium phosphate buffer pH 7.0 (60 : 40 w/w) (chromatographic system A). Quantification of undegraded VCR and VDS was based on peak height measurements. Further details can be found in Vendrig et al. (1988a).

The stability-indicating capability of the reversed-phase HPLC method was verified by chromatographic analysis of collected eluates of VCR and VDS peaks using non-modified silica gel as

stationary phase (LiChrosorb SI-60, particle size 10 μm; 300 × 3.9 mm i.d.) (Vendrig et al., 1988b). The mobile phase used in this system consisted of acetonitrile and buffer (85 : 15 w/w). The buffer contained 50 mM tetrabutylammonium bromide and 10 mM sodium dihydrogen citrate, adjusted to pH 3.0 with sodium hydroxide. The flow rate was 3.0 ml/min (chromatographic system B).

Results and Discussion

Chromatography

The chromatographic system designed for the degradation study of VBL (Vendrig et al., 1988a) was also suitable for the separation of VCR or VDS and their degradation products. Typical chromatograms are shown in Figs. 2 and 3 for VCR and VDS, respectively. For the analysis of VCR at high pH values a slight modification of the composition of the mobile phase (from 60 : 40 to 57.5 : 42.5 w/w methanol/buffer) was necessary to obtain sufficient resolution between VCR and one of the degradation products (peak 3 in Fig. 2E). The HPLC method used (system A) was stability-indicating. This was tested by chromatographic analysis of the eluates of the VCR and VDS peaks in partly degraded samples using chromatographic system B. Also the eluate of blank buffer was analysed. Compared to a chromatogram of the identically treated blank buffer no peaks beside the VCR and VDS peaks themselves were seen in the chromatograms of the eluates of the undegraded VCR and VDS peaks. In addition, we determined the ratio of heights of undegraded VCR and VDS peaks at 254 and 280 nm during the course of several degradation experiments. The ratio remained constant during the whole process of degradation, indicating that no degradation products with a different UV spectrum than VCR or VDS co-elute with undegraded VCR and VDS.

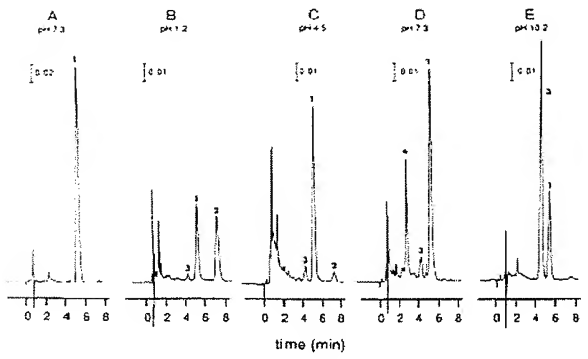

Fig. 2. Chromatograms of VCR before and during degradation at 80 °C at different pH values ($\mu = 0.3$). A: 0.035 M phosphate buffer, $t = 0$ h. B: 0.060 M perchloric acid, $t = 0.5$ h. C: 0.035 M phosphate buffer, $t = 72$ h. D: 0.035 M phosphate buffer, $t = 7.2$ h. E: 0.035 M carbonate buffer, $t = 0.5$ h. Peak 1: vincristine. Peaks 2–4: degradation products.

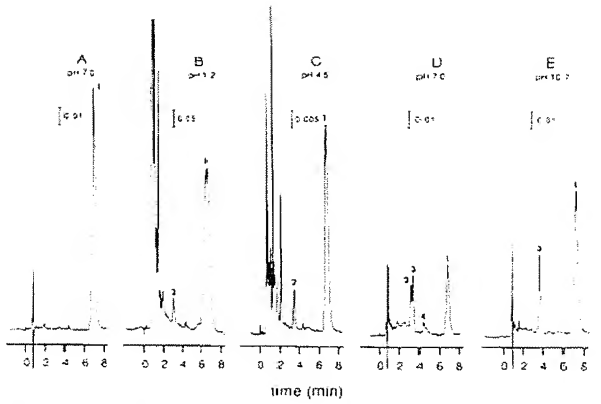

Fig. 3. Chromatograms of VDS before and during degradation at 80 °C at different pH values ($\mu = 0.3$). A: 0.035 M phosphate buffer, $t = 0$ h. B: 0.060 M perchloric acid, $t = 201.5$ h (no sodium acetate added). C: 0.035 M phosphate buffer, $t = 32.1$ h. D: 0.035 M phosphate buffer, $t = 11$ h. E: 0.035 M carbonate buffer, $t = 3.5$ h. Peak 1: vindesine. Peaks 2–4: degradation products.

Degradation products

Vincristine. Fig. 2A shows a chromatogram of VCR before degradation. Below pH 1.5 mainly one peak with a greater retention time than VCR emerges in the HPLC chromatograms (Fig. 2B, peak 2). Desacetylvincristine (DVCR) is also formed at pH 1.2 (peak 3), but only in minor quantities, judged from the peak heights. DVCR was identified by comparison of the elution volume of peak 3 with the reference. From pH 2 up to 4 some small peaks eluting with the solvent front were noticed. HPLC analysis of degraded solutions at pH values above 4 showed various peaks

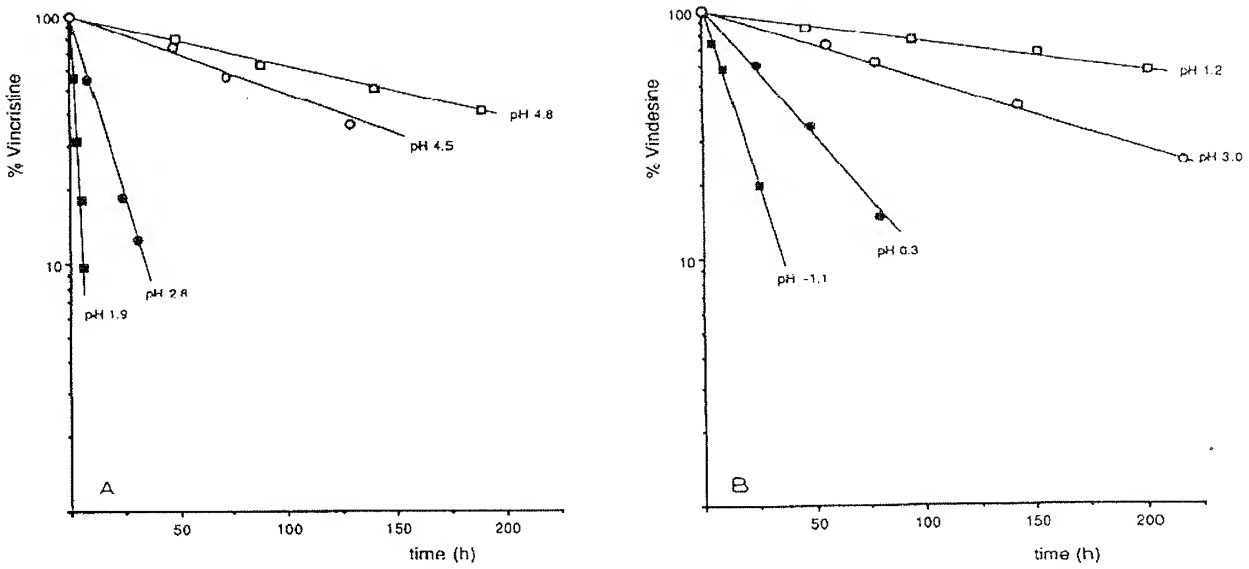

Fig. 4. Semilogarithmic pseudo-first-order plots for the degradation of VCR (A) and VDS (B) at 80 °C at different pH values ($\mu = 0.3$).

with a shorter retention time than VCR (Fig. 2D). DVCR (Fig. 2, peak 3) was formed to a greater or lesser extent in the whole pH region investigated. The shape of peak 3 (Figs. 2C and 2D, peak 3) indicated the presence of a compound co-eluting with DVCR when degradation mixtures with pH 3–8 were analysed. In the pH region 6.5–8, peak 4 (Fig. 2D) was formed, apart from peak 3. Only some very small peaks were seen before this. At pH values over 9, DVCR was the main detectable degradation product. The structures of all degradation products have not been elucidated yet.

Vindesine. Fig. 3A shows a chromatogram of VDS before degradation. HPLC analysis of degraded VDS solutions (pH < 1) showed a number of peaks eluting with the solvent front and a peak with a retention time of about 3 min (Fig. 3B, peak 2). At pH values above 1, a complex pattern of compounds which elute with the solvent front appeared (Fig. 3C and D). Compound 2 was also formed at these pH values but at a later stage of the degradation process. Around pH 4 a few separated peaks were seen in the front. In the pH region 5.5–7, only a few products were seen at the beginning, but peak 2 was formed to the greater extent. In the range pH 7–9, compound 3 was formed, which had almost the same retention time, but not identical, as compound 2. Also a degradation product with a retention time between that of compound 3 and VDS was observed (Fig. 3D, peak 4). At higher pH values only product 3 appeared in the chromatograms beside VDS.

Kinetics

This kinetic study was performed at 80 °C since the degradation rates of VCR and VDS at lower temperatures were too slow to obtain reliable kinetic data. Furthermore, the kinetics of VBL had previously been studied at 80 °C (Vendrig et al., 1988a). By using the same reaction conditions it is possible to compare the degradation kinetics of the 3 Vinca alkaloids.

Order of reaction

In buffers, the degradation of VCR and VDS exhibited a linear relationship between the natural logarithm of the concentration of undecomposed Vinca alkaloid and time (Fig. 4A and B). This pseudo-first-order kinetic behaviour was followed over at least 3 half-lives. The observed pseudo-

first-order rate constants (k_{obs}) were calculated from Eqn. 1:

$$\ln[VA]_t = \ln[VA]_0 - k_{obs} \cdot t \quad (1)$$

where $[VA]_t$ and $[VA]_0$ are the remaining and initial concentration of Vinca alkaloid at time t and 0, respectively.

Standard deviation in k_{obs}

For VCR, the mean k_{obs} and the standard deviation (S.D.) in k_{obs} were determined at pH 1.0 (perchloric acid, $\mu = 0.1$) and at pH 6.4 (0.050 M, $\mu = 0.3$). The mean $k_{obs} \pm$ S.D. values were $6.6 \pm 0.3 \times 10^{-4}$ s^{-1} ($n = 6$) and $7.6 \pm 0.7 \times 10^{-6}$ s^{-1} ($n = 6$), respectively. For VDS the mean k_{obs} and the S.D. in k_{obs} were determined at pH 0.5 (perchloric acid, $\mu = 0.3$) and pH 5.3 (0.050 M, $\mu = 0.3$). The mean $k_{obs} \pm$ S.D. values were $2.5 \pm 0.3 \times 10^{-6}$ s^{-1} ($n = 5$) and $7.8 \pm 0.5 \times 10^{-6}$ s^{-1} ($n = 6$), respectively. The values of the standard deviations were normal for this type of experiments. All other rate constants were determined in duplo.

Influence of buffer concentration

The influence of the concentration of sodium phosphate was studied in the range 0.005–0.050 M. The ionic strength was adjusted to 0.3 by addition of calculated amounts of sodium chloride. For VCR the buffer influence was studied at pH 4.5 and pH 9.2. For VDS pH 4.5, 6.2 and 8.2 were chosen for this purpose. Only for VDS at pH 6.2 a slight influence of the buffer concentration was found. The relation between the k_{obs} and the buffer concentration was not linear (Fig. 5). At the other pH values investigated, the degradation rates of VCR and VDS appeared independent of the buffer concentration. Table 1 documents representative data.

Influence of ionic strength

The influence of the ionic strength was investigated in the range 0.1–0.4 at pH 1.8, 4.5 and 9.2 for VCR and at pH 1.2, 3.5, 4.5, 6.2 and 8.2 for VDS. The degradation rates proved to be independent of the ionic strength at all pH values investigated. Representative data are listed in Table 2.

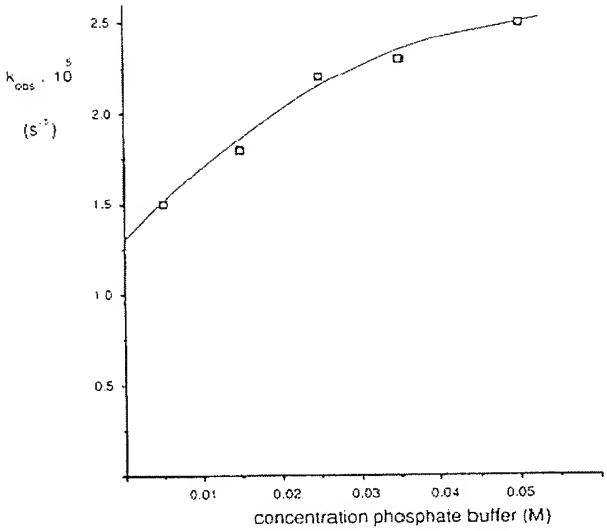

Fig. 5. Influence of the phosphate concentration on k_{obs} for the degradation of VDS at pH 6.2.

Influence of temperature

The influence of temperature on the degradation rates of the Vinca alkaloids was studied in the range from 60 to 80 °C at pH 1.2, 3.5, 5.2, 7.0 and 8.2. The activation energies (E_a) and the frequency

TABLE 1

The influence of the concentration of phosphate buffer on the k_{obs} for the degradation of VCR and VDS at different pH values ($\mu = 0.3$; temperature 80 °C)

pH	[buffer] (M)	k_{obs} (s^{-1})	pH	[buffer] (M)	k_{obs} (s^{-1})
Vincristine					
4.4	0.005	2.4×10^{-6}	9.2	0.005	1.2×10^{-4}
4.4	0.015	2.1×10^{-6}	9.2	0.015	1.9×10^{-4}
4.4	0.025	2.2×10^{-6}	9.2	0.025	1.5×10^{-4}
4.4	0.035	2.4×10^{-6}	9.2	0.035	1.5×10^{-4}
4.4	0.050	2.8×10^{-6}	9.2	0.050	1.6×10^{-4}
Vindesine					
4.4	0.005	8.4×10^{-6}	8.2	0.005	3.6×10^{-5}
4.4	0.015	7.6×10^{-6}	8.2	0.015	4.1×10^{-5}
4.4	0.025	8.3×10^{-6}	8.2	0.025	n.d.
4.4	0.035	8.9×10^{-6}	8.2	0.035	3.6×10^{-5}
4.4	0.050	8.2×10^{-6}	8.2	0.050	3.6×10^{-5}

TABLE 2

The influence of the ionic strength on the k_{obs} for the degradation of VCR and VDS at different pH values (concentration phosphate buffer 0.035 M, temperature 80°C)

pH	μ	k_{obs} (s^{-1})	pH	μ	k_{obs} (s^{-1})
Vincristine					
1.8	0.1	1.6×10^{-4}	4.5	0.1	2.2×10^{-6}
1.8	0.2	1.9×10^{-4}	4.5	0.2	2.5×10^{-6}
1.8	0.3	1.4×10^{-4}	4.5	0.3	2.1×10^{-6}
1.8	0.4	1.6×10^{-4}	4.5	0.4	2.6×10^{-6}
Vindesine					
3.5	0.1	7.2×10^{-6}	6.1	0.1	2.6×10^{-5}
3.5	0.2	7.3×10^{-6}	6.1	0.2	2.6×10^{-5}
3.5	0.3	6.0×10^{-6}	6.1	0.3	2.3×10^{-5}
3.5	0.4	6.1×10^{-6}	6.1	0.4	2.4×10^{-5}

factors (A) were calculated from the Arrhenius equation (Eqn. 2):

$$\ln k_{obs} = \ln A - (E_a/RT) \qquad (2)$$

in which R represents the molar gas constant and T the absolute temperature (K). Table 3 documents the results of these experiments in terms of activation energies and frequency factors.

Influence of pH

The degradation experiments were carried out in buffered solutions. Taking the results of the VBL study into account, we assumed that both the concentration of the buffer ions and the ionic strength of the solution had no influence on the degradation rates of VCR and VDS. This was substantiated by the experiments at selected pH values (1.2, 3.5, 4.5, 6.2, 8.2 and 9.2) whereby the buffer concentration and ionic strength were varied and no influences were noticed. Therefore, the k_{obs} values used for the construction of the pH-profile were the mean of all data determined at a specific pH ($n = 2$, 4 or 8). Only the k_{obs} value for VDS at pH 6.2 was extrapolated at zero buffer concentration.

TABLE 3

Activation energies (E_a) and frequency factors (A) for the degradation of VCR and VDS at various pH values (80°C)

pH	Vincristine		Vindesine	
	E_a (kJ·mol^{-1})	A (s^{-1})	E_a (kJ·mol^{-1})	A (s^{-1})
1.2	62	1×10^{6}	124	3×10^{12}
3.5	84	9×10^{6}	114	5×10^{11}
5.2	73	4×10^{5}	108	1×10^{11}
7.0	106	9×10^{10}	91	9×10^{8}
8.2	116	9×10^{12}	76	6×10^{6}

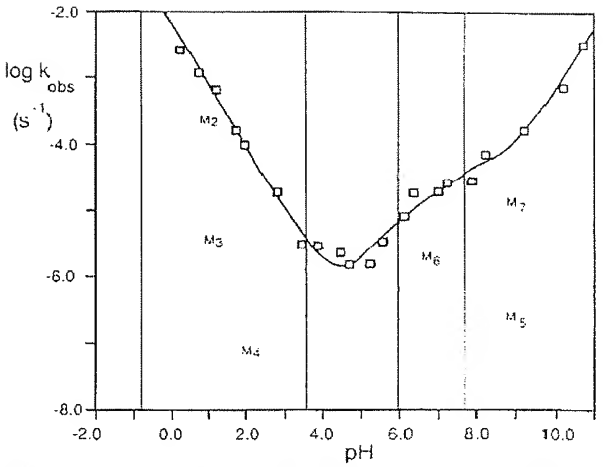

Fig. 6. Log k_{obs}–pH profile for the degradation of VCR at 80°C, fitted according to a 4 pK_a model.

Regression analysis of the log k_{obs}–pH rate profiles

Vincristine. The pH–rate profile of VCR (Fig. 6) did not exhibit pronounced inflection points from which all 4 pK_a values of VCR could be calculated with sufficient precision. Non-linear regression analysis using the mathematical equation derived by Van Der Houwen et al. (1988) was therefore not possible. With the k_{obs} values determined, an infinite number of combinations of macro-reaction constants and dissociation constants existed, which all gave an acceptable correlation between the model and the experimental values. Calculation of the macro-reaction constants would be possible after accurate determination of the protolytic dissociation constants using other methods. Since this had to be done at the temperature of the kinetic study (80°C) and because of the presence of overlapping ionization constants, these experiments would be very com-

TABLE 4

Macro-reaction constants for the degradation of VCR and VDS, ionization constants and pK values at a temperature of 80 °C. The pKa values of VCR are the mean of the pK_a values of VBL (Vendrig et al., 1988a) and VDS

	Vincristine	Vindesine
M_1	a	a
M_2	5.4×10^{-2} s^{-1}	7.6×10^{-5} s^{-1}
M_3	3.2×10^{-5} $M\cdot s^{-1}$	a
M_4	7.8×10^{-10} $M^2\cdot s^{-1}$	1.1×10^{-8} $M^2\cdot s^{-1}$
M_5	1.8×10^{-14} $M^3\cdot s^{-1}$	1.1×10^{-13} $M^3\cdot s^{-1}$
M_6	1.6×10^{-21} $M^4\cdot s^{-1}$	2.2×10^{-22} $M^4\cdot s^{-1}$
M_7	1.5×10^{-30} $M^5\cdot s^{-1}$	a
K_1	6.3	5.6
K_2	2.5×10^{-4}	3.0×10^{-4}
K_3	1.0×10^{-6}	1.7×10^{-6}
K_4	2.0×10^{-8}	3.4×10^{-9}
pK_1	−0.8	−0.8
pK_2	3.6	3.5
pK_3	6.0	5.8
pK_4	7.7	8.5

[a] Contribution not significant.

plicated with presumably poor results and, therefore, were omitted. Furthermore, the precision of the kinetic measurements themselves was limited. Therefore we concluded it was not possible to calculate the macro-reaction and dissociation constants for VCR with the k_{obs} values measured. The constants listed in Table 4 are the result of a fit using the mean of the pK_a values calculated for VBL and VDS as pK_a values for VCR. This reduces the possibilities for the macro-reaction constants considerably.

In Figs. 6 and 7 the squares represent the experimental k_{obs} values. The dark line is the result of the fit using the constants listed in Table 4. The light lines represent the contribution of the separate macro-reaction constants to k_{obs}. The fit in Fig. 6 had been made by taking the results of VBL and VDS into account. As can be seen from Fig. 6, the model calculated with estimated constants showed moderate correlation with the experimental values.

Vindesine. For VDS the macro-reaction constants and the pK_a values were calculated with the non-linear least-squares curve-fitting program,

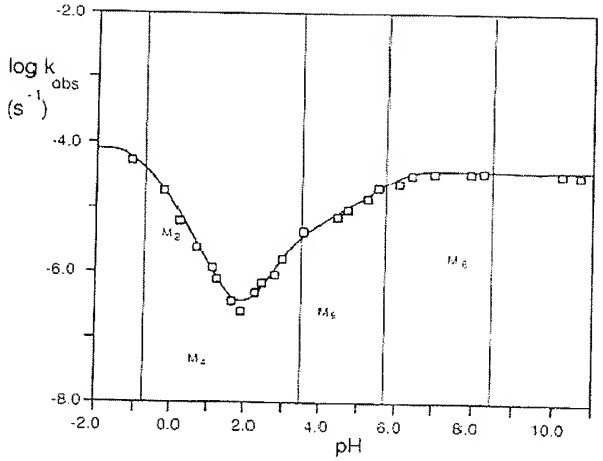

Fig. 7. Log k_{obs}–pH profile for the degradation of VDS at 80 °C, fitted according to a 4 pK_a model.

using an Olivetti microcomputer. In Table 4 the results of the optimal fit are presented. For VDS the calculated model showed a high correlation with the experimental values (Fig. 7). The slope in the pH region −0.5 to 1.7 was −1. Between pH 1.7 and pH 1.9 the degradation rate was independent from the pH. From pH 1.9 to pH 2.8 the slope was +1. Above pH 7.0 the degradation rate was independent of the pH.

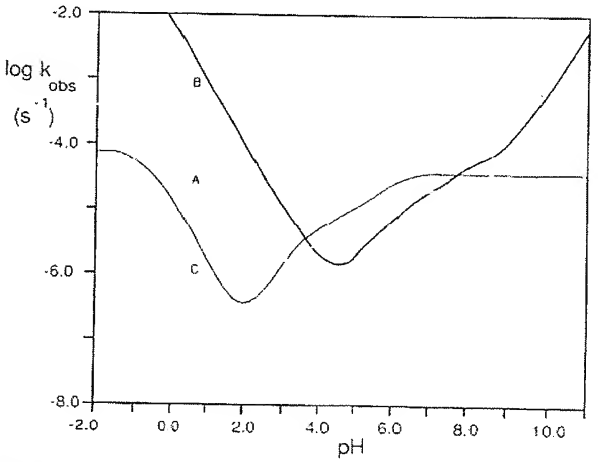

Fig. 8. Log k_{obs}–pH profiles for the degradation of VBL (A), VCR (B) and VDS (C) at 80 °C (fitted according to a 4 pK_a model).

A comparison of the overall stability of vinblastine, vincristine and vindesine

The log k_{obs}–pH profiles for VBL (data obtained from an earlier study: Vendrig et al., 1988a), VCR and VDS have been combined in Fig. 8. These curves reveal that the 3 Vinca alkaloids possess distinct differences in terms of overall chemical stability in aqueous solutions while the drugs have minor structural differences. Substitution of the N-1 aldehyde function in VCR by a methyl group in VBL results in a much more stable compound in solutions at pH < 4 and pH > 7. On the contrary, VBL is less stable than VCR in the intermediate pH region. To conclude, the N-1 substituent has a great impact on the overall chemical stability of these compounds. In alkaline solutions hydroxyl-catalyzed desacetylation constitutes the main degradation pathway for VBL and VCR. The absence of an acetyl group in VDS may, therefore, explain the totally different character of the pH-profile of this compound in alkaline solutions compared to the profiles of VBL and VCR. Full interpretation of the pH-profiles will only be possible after structure elucidation of the degradation products. This is under current investigation.

Conclusions

Vincristine is most stable in aqueous solutions between pH 3.5 and 5.6. For VDS this pH region is between pH 1.6 and 2.0. The longest degradation half-life is 136 h for VCR at pH 4.8 and 690 h for VDS at pH 1.9. For VBL the longest half-life is 121 h at pH 3.0. The concentration of phosphate buffer has no influence on the degradation rate in the range from 0.005 to 0.0050 M, except for VDS at pH 6.2. The ionic strength, adjusted with sodium chloride, has no effect in the range 0.1–0.4.

When the degradation kinetics of the 3 Vinca alkaloids are compared, VDS proves to be the most stable compound at pH 1.9. The stabilities of VCR and VBL are comparable to each other but the pH at which the compounds are most stable differs (pH 4.8 and 3.5, respectively).

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